Analytical Supercritical Fluid Chromatography and Extraction

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CHAPTER 1

INTRODUCTION

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1.1 Definition and Description of Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) may be defined as a form of chromatography (*i.e.*, a physical separation method based on the interaction of an analyte in a mobile phase with a stationary phase) in which the mobile phase is subjected to pressures and temperatures near or above the critical point for the purpose of enhancing the mobile phase solvating power. Typically, one or both parameters (*i.e.*, pressure and temperature) extend into the critical region during a chromatographic run. This definition encompasses other less defined forms of chromatography such as dense gas chromatography, hyperpressure gas chromatography, and near (or sub-) critical fluid chromatography. As shown in Figure 1.1, the use of supercritical solvents for chemical separations, including both chromatography and extraction, represents a unique niche in high pressure research.

There is more to the definition of SFC than is implied from the strict definition of a supercritical fluid. In fact, helium is supercritical in gas chromatography (GC) when pressures in the column are greater than 2.26 atm. Since under these conditions the whole length of the column is not supercritical (the column outlet is usually at atmospheric pressure) and the mobile phase has essentially no solvating power, this cannot be considered SFC. Furthermore, if one investigates the practical operating conditions of SFC, it will be obvious that many of the chromatographic analyses are started at pressures below the critical pressure, and occasionally at subcritical temperatures. In general, three conditions must be met to truly define SFC: (a) the mobile phase must always be at temperatures and pressures near or above their critical point, (b) the mobile phase must

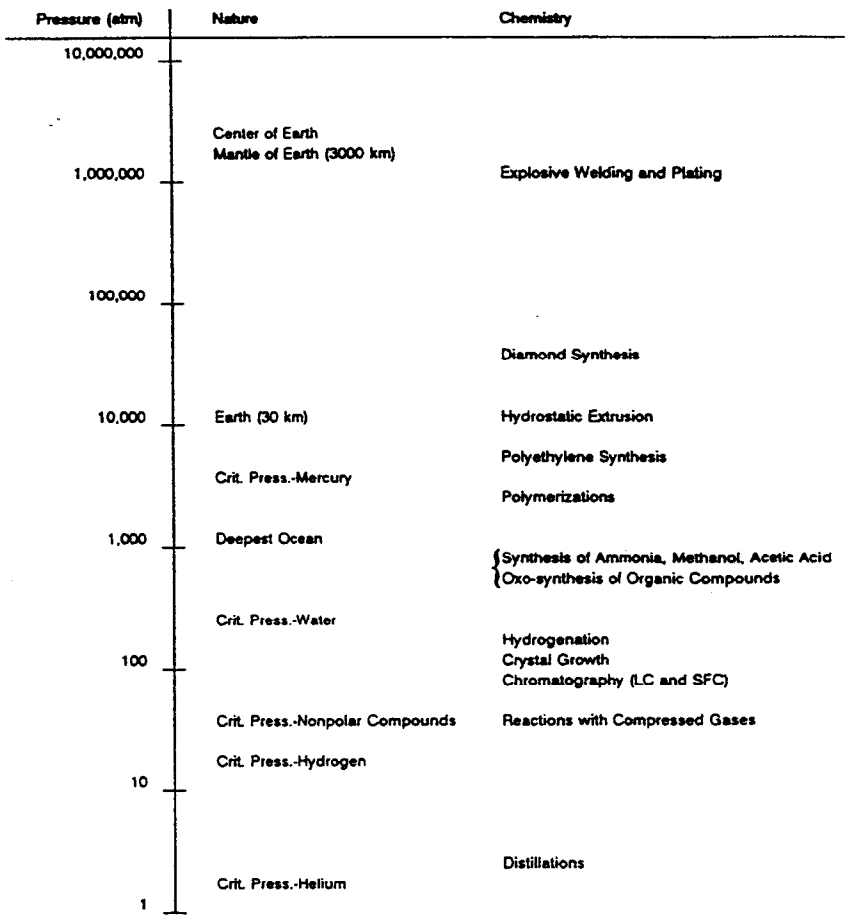

Figure 1.1. Pressure scale in nature and chemistry (according to similar compilations by Schneider[1] and Pilz[2]).

possess solvating power and, thus, be able to contribute to selectivity in the chromatographic process, and (c) the mobile phase must be subject to these conditions throughout the full length of the analytical column.

The nature of supercritical fluids is such that the mobile phase can easily be varied from gas-like to liquid-like. Based on judicious choice of mobile phase, this may lead to a variety of advantages for SFC over other separation techniques. Fluids with low critical temperatures permit operation at temperatures conducive to the analysis of thermally labile solutes and allow for greater stationary phase selectivity. The higher solvating ability of supercritical fluids

permits the analysis of high molecular mass, nonvolatile compounds. SFC is compatible with essentially all detectors commonly used in both gas chromatography (GC) and liquid chromatography (LC). Both open tubular and packed columns, with the advantages and disadvantages associated with each column type, are widely used. SFC has a variety of parameters (*i.e.*, pressure, density, temperature, mobile phase composition, and stationary phase composition) which can be manipulated to effect solute retention. Finally, the coupling of extraction methods or multidimensional systems with SFC is possible, as in other forms of chromatography.

SFC can be conveniently divided into two categories based on column type: open tubular and packed. The choice of column type is due not only to the obvious chromatographic differences (*e.g.*, sample capacity, resolving power, *etc.*), but also to differences in column pressure drop and volumetric flow, which impose different constraints upon the system. Packed columns for SFC can be classified into three types, borrowing from developments in LC: (a) conventional packed columns that typically are of 2-4.6 mm internal diameter (i.d.), (b) 1-mm i.d. "microbore" columns, and (c) packed capillaries that have diameters of 0.2-0.8 mm. Packing materials of 3-10 μm diameter are similar to those used in LC. Peaden and Lee[3] have shown that to achieve resolution comparable to capillary GC with 200-μm i.d. columns, open tubular columns for SFC must have internal diameters smaller than 100 μm. Hence, 50-μm i.d. columns are commonly used in open tubular column SFC, although research on smaller diameter columns is in process.[4] The SFC equivalent to GC "megabore" columns (*i.e.*, >100-μm i.d. for SFC) has not come into common use. Column considerations are addressed in Chapter 2.

Of course, it is the nature of the mobile phase which is unique to SFC. The properties of supercritical fluids (*e.g.*, diffusivity, density, viscosity, *etc.*) are intermediate between those of gases and liquids, and can be varied by subtle changes in the operational parameters (*i.e.*, pressure and temperature). It is these properties that are exploited in SFC. Gases, supercritical fluids, and liquids have been compared as chromatographic mobile phases,[5-7] and criteria for selecting suitable mobile phases for SFC have been specified. These considerations include: (a) critical pressure, (b) critical temperature, (c) dipole moment, (d) chemical interactions with the stationary phase, (e) chemical interactions with the analyte, (f) compatibility with the detection system, (g) compatibility with seals, tubing, and pumps, (h) environmental and safety considerations, (i) cost, and (j) purity.

To enhance the solvating power of the supercritical mobile phase for polar or high molecular mass analytes, miscible organic dopants or entrainers, known as modifiers, are commonly added to SFC mobile phases. The modifier may interact with the analyte, mobile phase, or stationary phase to influence solute retention. Three advantages have been noted[8] as accompanying the use of modifiers: (a) improvement in the solubility of low-volatility and polar solutes, (b) modification of the pressure-volume-temperature (PVT) behavior of the supercritical fluid, and (c) improvement in selectivity. Various mechanisms have been proposed[9] to account for the modifier effect in SFC. These are: (a) interaction of polar molecules with stationary phase active sites, (b) solute-modifier interactions forming stable species which favor the mobile phase, (c) short-range clusters formed between the modifier and the mobile phase, and (d) a decrease in interfacial tension between the mobile and stationary phases. SFC mobile phase considerations are discussed in depth in Chapter 3.

As with any emerging technology, a variety of terminology has evolved, much of which is confusing or inconsistent. Through either developing trends, correct language usage, or convenience, the terminology and units suggested in this paragraph are recommended. The term "isopycnic" has been suggested to denote constant density at constant pressure and temperature.[10,11] Isopycnic is derived from the Greek word "pycnos," meaning dense, and therefore it denotes a body having equal density at different points; for example, pycnometers are density measuring instruments. Two isopycnic states may be attained at different temperatures merely by changing the pressure. Pressure units of atmospheres (atm) or bars are convenient and, consequently, programming rates of atm (or bar) min^{-1} follow. Density units of g mL^{-1} are advocated with density programming rates of g mL^{-1} min^{-1}. Since nearly all density (and pressure) programming is done isothermally, the operational temperature should be noted. To remain consistent with LC terminology, the term "gradient elution" should be restricted to mobile phase compositional gradients. Use of the term "simultaneous density-temperature programming" should only be used when density corrections for changing temperature have been made. Otherwise, the term "simultaneous pressure-temperature programming" would be preferred. Binary mobile phase systems are usually expressed in terms of concentration of modifier in the primary (or bulk) fluid. Mole percent, rather than weight or volume percent, is the preferred concentration unit since interactions occurring with modifiers are on a molecular basis and not on a mass or volume basis. Systems with

more than two components should be specified as ternary, quaternary, *etc.*, mixtures.

One of the major advantages of SFC is the potential compatibility of the technique with the full array of detectors available for both GC and LC. As will be presented in Chapter 4, numerous different on-line detectors have been reported. The most popular SFC detection methods are the flame ionization detector (FID), ultraviolet absorption detector (UV), mass spectrometer (MS), and Fourier transform infrared spectrometer (FTIR).

A compilation of over 330 SFC chromatograms[12] was prepared for the 1988 Workshop on Supercritical Fluid Chromatography; a companion volume[13], with 350 chromatograms, was compiled for the 1989 meeting. These compilations represent the wide range of applications for which SFC is well-suited. Applications of SFC are wide-ranging, including drugs, chiral compounds, foods and natural products, fatty acids and derivatives, glycerides, steroids, biomolecules, explosives, pesticides and herbicides, fossil fuels and aromatic compounds, polymer additives, polymers, and miscellaneous chemicals and products. Analytical applications of SFC are extensively discussed in Chapter 7.

1.2 Historical Development of SFC

The phenomenon of the "critical state" has been known since 1822 when Cagnaird de la Tour noted[14] the lack of discontinuity (*i.e.*, disappearance of a meniscus) when passing between the gaseous and liquid states (see Table 1.1). Andrews' 1869 study[15] on CO_2 is considered to be the first systematic study of a gas-liquid critical point, although it has been claimed[16] that the general idea of the "critical state" was arrived at independently by Mendeleeff in 1861. Later, in 1879 and 1880, Hannay and Hogarth[17-19] published the first account of the enhanced solvating properties of supercritical fluids. Other investigators made similar observations at an early date.[20-22] In studying the solubility of cobalt chloride, ferric chloride, potassium bromide, and potassium iodide in supercritical carbonic acid[18], Hannay and Hogarth found a "perfect continuity of the liquid and gaseous states." Hannay summarized the findings by stating[19] that,

> The liquid condition of fluids has very little to do with their solvent power, but only indicates molecular closeness. Should this closeness be attained by external pressure instead of internal attraction, the result

Table 1.1. Chronological Listing of "Firsts" in the Development of SFC

Year	Event
1822	Phenomenon of "critical state" reported.
1869	First systematic study of critical region.
1879	Enhanced solubility in supercritical fluids reported.
1958	Use of supercritical fluids as chromatographic mobile phases suggested.
1962	Use of supercritical fluids as chromatographic mobile phase demonstrated.
1966	First published SFC chromatogram.
	Flame ionization detection in SFC reported.
1967	Ultraviolet absorbance detection in SFC reported.
1969	First report on use of mobile phase modifiers (*i.e.*, binary mobile phases).
	Differential refractometry detection in SFC reported.
1970	Initial use of pressure programming.
	Heat of adsorption detection in SFC reported.
1978	Mass spectrometric detection in SFC reported.
	First report on use of (negative) temperature programming.
	First report on use of simultaneous temperature-density programming.
1981	First report on use of capillary (open tubular) columns in SFC.
	Fluorometric detection in SFC reported.
	First report on use of mobile phase compositional gradient programming.
1982	Hewlett-Packard introduces SFC instrumentation at Pittsburgh Conference.
1983	Thermionic (nitrogen-phosphorus) and Fourier transform infrared detection in SFC reported.
1985	First report on multidimensional SFC.
	First report on coupling supercritical fluid extraction to SFC (on-line).
	First report on chiral separation by SFC.
1986	Capillary SFC instrumentation introduced at Pittsburgh Conference.
	Flame photometric and ion mobility detection in SFC reported.
	First report of solute derivatization in SFC analysis.
1987	Photoionization, light scattering, and inductively coupled plasma detection in SFC reported.
	Use of reverse micelles as SFC mobile phase demonstrated.
1988	First report on use of ion-pairing agents in SFC.
	Hydrogen atmosphere flame ionization, microwave induced plasma, thermal energy analysis, redox chemiluminescence, nuclear magnetic resonance, radiofrequency plasma, and electron capture detection in reported.
	Journal of Supercritical Fluids, first journal devoted entirely to supercritical measurements and applications, introduced.
1989	Sulfur chemiluminescence and supersonic jet spectroscopy detection in SFC reported.

> is that the same or even greater solvent power is obtained. . . . The gas must have a certain density before it will act as a solvent, and when its volume is increased more than twice its liquid volume, its solvent action is almost destroyed.

Considerable time passed, however, before this basic knowledge was utilized for extraction or for chromatography, although both of these processes occupied a central role in the separation of substances. Early reports of extractions with supercritical fluids[23,24] have eventually led to large scale extraction processes.

The first documented suggestion of what is now called SFC was made in 1958 in a notarized and witnessed private note by Lovelock, then at Yale University, who wrote down his idea about the extension of GC to nonvolatile ionic compounds using gases in their supercritical state as mobile phases, such as water, ammonia, sulfur dioxide, and hydrogen fluoride.[25,26]

In 1961, the idea of using dense gases for the transport of nonvolatile substances through a chromatographic column was independently conceived and reduced to practice by Klesper *et al.*[27] in a simple chromatographic apparatus using porphyrins as the eluates. The method was then called "high pressure gas chromatography" instead of SFC.

Before the first SFC instrument was built, it was ascertained that GC could not be applied to the porphyrins which were of interest to Corwin's group at Johns Hopkins University. Klesper designed an experimental apparatus which was built and put into operation in 1961. The design was very simple, but it was only intended to show the feasibility of the approach. A diagram of this apparatus is shown in Figure 1.2. No mechanical pump or elaborate detector was used. Instead, a heated pressure vessel provided a mobile phase at elevated pressures, and a spiral of copper tubing functioned as a final heat exchanger to reach the desired temperature. The column was contained in a glass high pressure gauge tube which allowed observation of the column and the colored bands of porphyrins which moved down the column. Chlorofluoroalkanes were used as mobile phases to move and separate bands of etioporphyrin II, Ni etioporphyrin II, and Ni mesoporphyrin IX dimethylester on the column. The chlorofluoroalkanes were used as mobile phases because of their low flammability and physiological inertness.

While the group of Corwin published their work on porphyrins, Giddings *et al.* were studying the general aspects of pressure induced equilibrium shifts in chromatography[28] and dense GC of nonvolatile species,[29,30] including size exclusion SFC.[31] Another group which contributed even earlier and very successfully to the development of

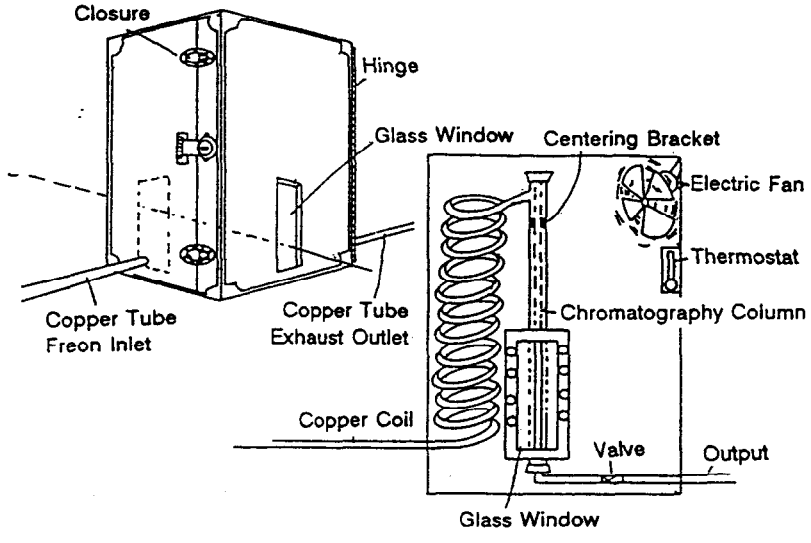

Figure 1.2. Original SFC apparatus used by Klesper.

SFC, was that of Sie and Rijnders at the Shell Laboratories in Amsterdam. This group performed in-depth studies on the effect of pressure on partition coefficients,[32] as well as the effect of mobile phase velocity and particle size on column plate height and permeability.[33] Moreover, they performed SFC both in the gas-liquid and gas-solid mode,[34,35] and investigated the use of porous polymeric stationary phases.[36]

In 1967, Sie and Rijnders[37] were the first to use the term "supercritical fluid chromatography" for this new chromatographic technique, and suggested the use of mobile phase pressure programming. Later, Jentoft and Gouw were the first to employ pressure programming[38] and mobile phase modifiers[39] (*i.e.*, methanol in *n*-pentane) to control retention in SFC. These workers published useful early reviews on SFC.[40,41] Figure 1.3 shows the temperature-pressure regions explored in these early SFC studies.

In 1971, Novotny *et al.* published an important paper concerning the effects of temperature, pressure, eluent composition, flow rate, and type of stationary phase on capacity factors.[42] Physicochemical measurements by SFC, particularly with respect to thermodynamics and mass transport, were reported by Bartmann and Schneider.[43] Schneider and coworkers continued in the years that followed to place emphasis on physicochemical aspects, with studies on the density dependence of capacity factors,[44] binary diffusion

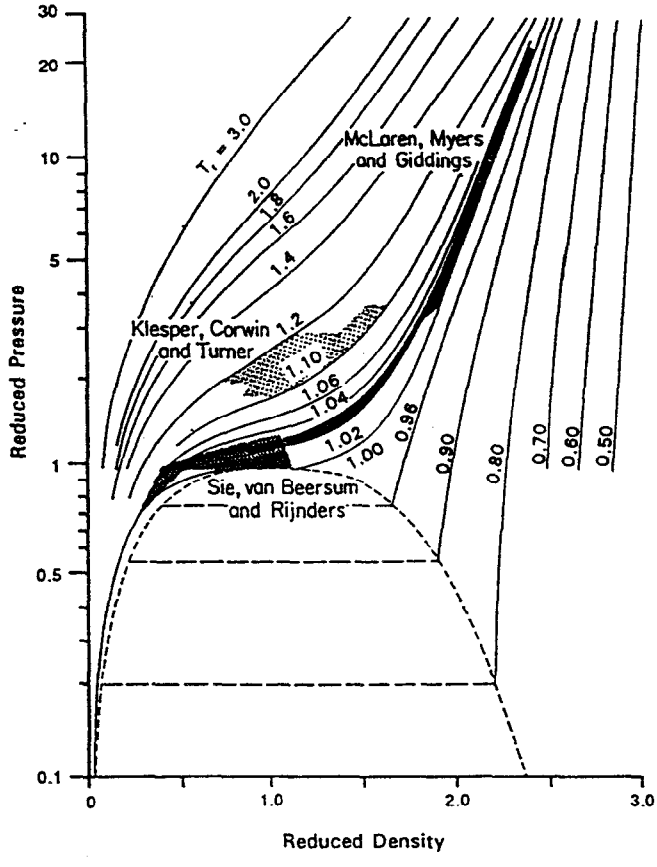

Figure 1.3. CO_2 pressure as a function of density at different temperatures, expressed in terms of reduced parameters. The area below the dotted line represents the two-phase gas/liquid equilibrium region. The indicated areas refer to conditions at which these researchers performed their experiments during the initial studies of SFC (reprinted with permission from Ref. 29).

coefficients,[45] and partial molar volumes.[46] More practical investigations were conducted by Rogers and coworkers, including the separation of oligomers by pressure programming, temperature programming, and modifiers, using both normal and reversed stationary phases.[47-49] Hybrid techniques made their first appearance in SFC when SFC was interfaced to MS by Randall and Wahrhaftig.[50]

The most rapid growth in SFC occurred during the 1980s. Packed column SFC became commercially available in 1982 with the introduction of a modified Hewlett-Packard liquid chromatograph.

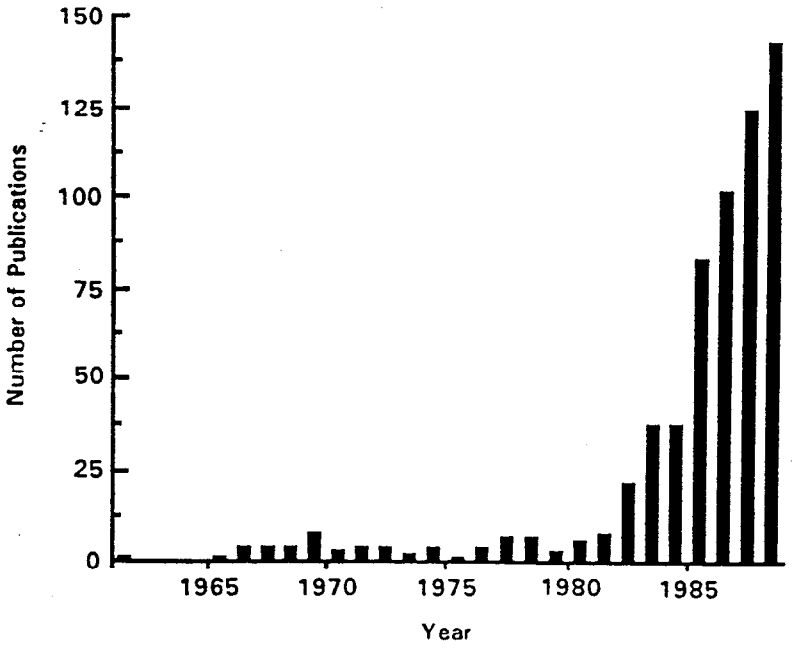

Figure 1.4. Frequency of SFC publications from 1962 to 1989.

The research groups of Novotny and Lee successfully introduced capillary SFC in 1981,[51] which increased the momentum for the development and general acceptance of the technique, and resulted in capillary instrumentation on the market by 1986. This growth is illustrated in Figure 1.4 which shows the number of papers on SFC published each year since its inception. Numerous detectors have been evaluated and modified for use with supercritical fluids, the column technologies for both packed and open tubular columns have advanced, multidimensional methods including on-line supercritical fluid extraction (SFE) have been reported, and the technique has been applied to a wide range of compound types. Detailed discussions of these developments are given in the following chapters.

1.3 History of Analytical SFE

SFE is a technique, like SFC, that employs a supercritical fluid phase to effect the solubilization and separation of solutes. It should be apparent that SFE is closely allied with SFC. In fact, SFE is embodied within SFC theory, since chromatographic plate theory[52] may be interpreted as a series of solute partitions or extractions

between the mobile and stationary phases. This close correspondence between the two techniques was historically noted by early researchers in SFC, who found that chromatographic partition coefficients and capacity factors were dependent on the nature of the carrier gas[53] and the column operating pressure.[32] The possibility of enhancing the migration of high molecular mass solutes was noted by Giddings in 1964[54] and amply demonstrated by his research group in the late 1960s.[29,30] Nonetheless, the analytical potential of SFE lay dormant over the next decade and a half, until the mid-1980s.

Whereas the use of supercritical fluid media for performing analytical scale extractions is a recent development, the occurrence of supercritical fluid phenomena can be documented to the early 19th century.[14] Historically, as previously described, the solvent properties of supercritical fluids were initially reported by Hanney and Hogarth,[17-19] and an excellent review of these early studies is provided by Booth and Bidwell.[55] Unfortunately, the extraction potential of these fluids remained unrecognized for many years, although the phenomena of "retrograde condensation" of solutes from compressed gas solutions was recognized very early in the field of petroleum engineering.[56] The solvent properties of liquefied gases were also explored throughout the 1940s and 50s, and the pioneering research of Francis,[57] who compiled an extensive collection of ternary phase diagrams for liquid CO_2 with organic and inorganic compounds and estimated the solubilities of 261 compounds in near-critical CO_2, deserves particular mention. However, it was not until the appearance of a key patent by Zosel[23] that the potential of SFE as a processing technique became apparent. Since this time, there has been a steady growth in the number of applications of SFE in the chemical engineering field, leading recently to the construction of several plants designed to decaffeinate coffee and to process hops and spices. A recent book by McHugh and Krukonis[58] presents the chemical engineering perspective of SFE and is highly recommended as a complementary text.

By contrast, analytical SFE differs from preparative-scale SFE in that the levels of solutes dissolved in the supercritical fluid medium are quite low compared to levels normally encountered in preparative SFE. In addition, the apparatus used to perform the extraction is miniaturized compared to any preparative-scale extractors. This, in part, is due to the frequent coupling of analytical SFE with chromatographic instrumentation in what has become known as "on-line SFE." By contrast, SFE may also be used independently of any other analytical technique, and in this "off-line" mode, may approach a scale that is equivalent to bench-scale preparative units.[59]

In its simplest form, a supercritical fluid extractor consists of a source of pressurized extraction fluid, an extraction cell, and a collection device for isolating the extract. Traditional sources of supercritical fluids have consisted of tanks filled with pressurized gas or semiliquefied gas that is delivered to the extraction cell *via* pumps or compressors. The choice of the latter device is partly based on whether one desires to deliver the fluid in its liquid or gaseous state. There are many different configurations for extraction cells, and these options are discussed in Chapter 5. Collection of the extract is usually accomplished by reducing the pressure on the fluid stream as it exits the extraction cell; however, phase separation of the dissolved solute from the supercritical fluid may also be achieved by changing the temperature of the exiting fluid. In on-line SFE, ancillary devices, such as sorbent cartridges or retention gaps, may be used to focus the solutes after extraction in preparation for chromatographic separation.

From a historical perspective, it is somewhat difficult to precisely identify a specific study or individual that can be credited with inventing analytical-scale SFE. This is partly due to the simultaneous development of SFE in several technical disciplines, as well as the lack of definition as to what really constitutes "analytical SFE." However, there is little doubt that the efforts of Stahl and Schilz in 1976[60] to combine SFE with thin-layer chromatography demonstrated the considerable potential of the technique for analytical studies.

1.4 Definition of a Supercritical Fluid

The strict definition of a supercritical fluid can be visualized by reference to the pressure/temperature phase diagram shown in Figure 1.5. This phase diagram shows the relationship of the gas, liquid, and solid states of a substance as a function of temperature and pressure. The critical point is defined by a critical temperature (T_c) and a critical pressure (P_c) above which (as indicated by the dotted line in Figure 1.5) the substance is neither a gas nor a liquid, but possesses properties of both.

Supercritical fluids may be defined from a practical viewpoint as gases that are at temperatures usually above their critical temperatures and that are compressed to pressures (or densities) at which "liquid-like" interactions become significant. The combination of physical properties (*e.g.*, viscosity and diffusivity) with variable

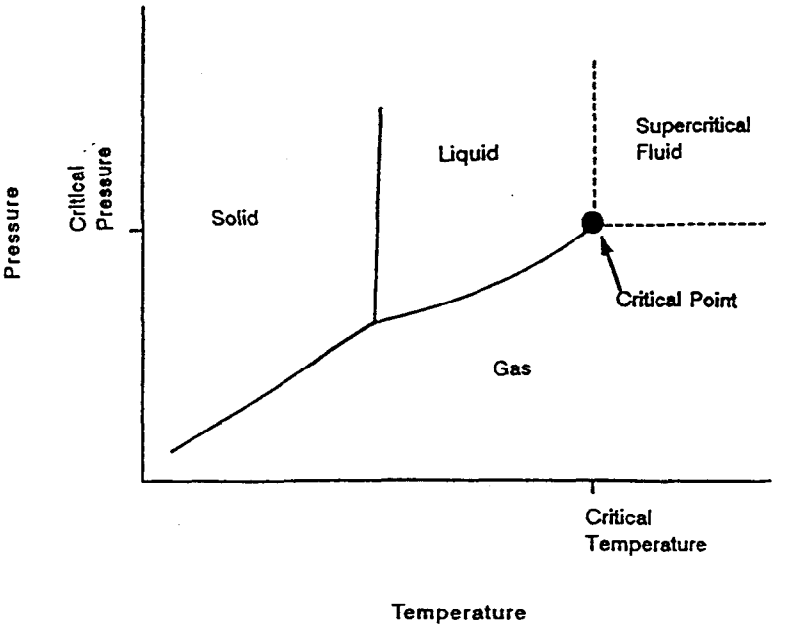

Figure 1.5. Pressure/temperature phase diagram of a substance.

solvating power provides the basis for the advantages of SFC and related analytical techniques. The physical properties of a supercritical fluid are variable between the limits of a normal gas and those of a liquid by control of pressure and temperature. Typically, supercritical fluids are used at densities ranging from 0.1 to 0.8 of their liquid density, and practical pressures for applications range from less than 50 atm to more than 500 atm. Under these conditions, the diffusion coefficients of supercritical fluids are substantially greater than those of liquids. For example, the diffusivity of supercritical CO_2 varies between 10^{-4} and 10^{-3} cm^2 s^{-1} over the range of conditions usually utilized, whereas liquids typically have diffusivities of less than 10^{-5} cm^2 s^{-1}. Similarly, the viscosities of supercritical fluids mirror their diffusivities, and are typically 10 to 100 times lower than for liquids.[61] These more favorable physical properties provide the advantages of supercritical fluids in chromatography and extraction applications.

Figure 1.3 shows the pressure-density relationship for CO_2 in terms of reduced parameters (*e.g.*, pressure, temperature, or density divided by the appropriate critical parameter), including the two-phase vapor-liquid region. This relationship is generally valid for most single-component systems. The isotherms at several reduced

temperatures show the variations in density that can be expected with changes in pressure. Thus, the density of a supercritical fluid will be typically 10^2 to 10^3 times greater than that of a gas at ambient temperatures. Consequently, molecular interactions increase due to shorter intermolecular distances. Table 1.2 gives the critical parameters for a number of common and potentially useful supercritical solvents.

For a neat supercritical fluid, and for very dilute supercritical fluid solutions relevant to chromatographic separations, the relationships between pressure, temperature, and density can be estimated with reasonable accuracy from equations of state, except near the critical point. However, it is often necessary to use a binary fluid mixture to obtain enhanced solvating power. The phase behavior of binary systems is highly varied and much more complex than in single-component systems, and has been well described in the literature for only selected model systems.[58] Six basic types of phase behavior have been identified, with the simplest type characterized

Table 1.2. Physical Parameters of Selected Supercritical Fluids

Fluid	Dipole moment (debyes)[a]	T_c (°C)[a]	P_c (atm)[a]	ρ_c (g mL^{-1})[a]	ρ_{400} (g mL^{-1})[b]	ρ_l (g mL^{-1})[a,c]
CO_2	0.00	31.3	72.9	0.47	0.96	0.71 (63.4 atm)
N_2O	0.17	36.5	72.5	0.45	0.94	0.91 (0°C)
						0.64 (59 atm)
NH_3	1.47	132.5	112.5	0.24	0.40	0.68 (-33.7°C)
						0.60 (10.5 atm)
n-C_5	0.00	196.6	33.3	0.23	0.51	0.75 (1 atm)
n-C_4	0.00	152.0	37.5	0.23	0.50	0.58 (20°C)
						0.57 (2.6 atm)
SF_6	0.00	45.5	37.1	0.74	1.61	1.91 (-50°C)
Xe	0.00	16.6	58.4	1.10	2.30	3.08 (111.75°C)
CCl_2F_2	0.17	111.8	40.7	0.56	1.12	1.53 (-45.6°C)
						1.30 (6.7 atm)
CHF_3	1.62	25.9	46.9	0.52	1.15	1.51 (-100°C)

[a]Data taken from Refs. 62 and 63.

[b]The density at 400 atm (ρ_{400}) and $T_r = 1.03$ was calculated from compressibility data.[64]

[c]Measurements were made under saturated conditions if no pressure is specified or were performed at 25°C if no temperature is specified.

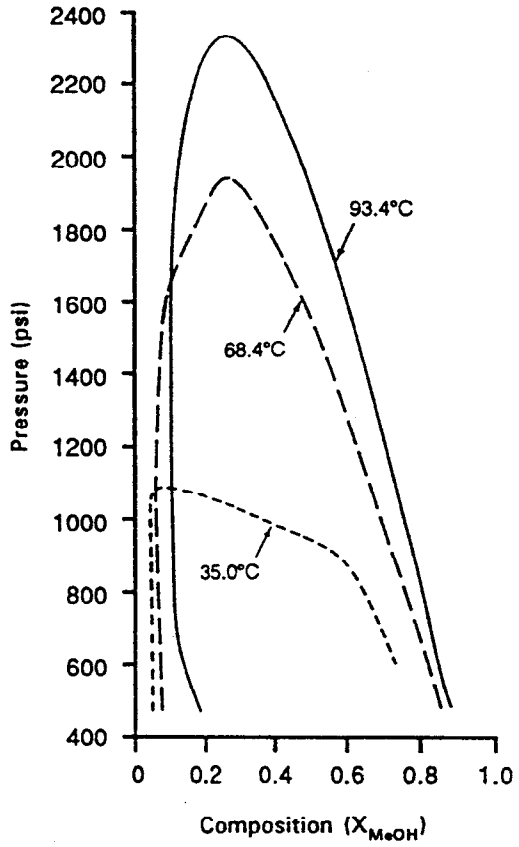

Figure 1.6. CO_2/methanol pressure composition vapor-liquid envelopes at various temperatures (reprinted with permission from Ref. 66).

by a continuous mixture curve for pressure and temperature conditions over the composition range of the two components. Examples of binary fluid systems of this type are CO_2 with isopropanol or methanol, and propane with isopropanol. A detailed discussion of the more complex types of binary fluid mixtures and the phase behavior of these systems can be found elsewhere.[65] The phase behavior of CO_2-methanol is shown in Figure 1.6, which gives the pressure-composition vapor-liquid envelopes for three temperatures.[66,67] The single-phase supercritical regions are above the envelopes, and the regions within the envelopes correspond to two-phase subcritical mixtures at their respective temperatures.

1.5 Characteristics of Supercritical Fluids Relevant to Separation Science

To exploit the chromatographic advantages of SFC, it is essential that the fluid mixtures used for mobile phases be selected such that they can be mixed and pumped as a single phase, preferably at ambient temperatures. Proper operating conditions must also be chosen to give a single-phase supercritical fluid, and care must be taken to avoid entering a two-phase region when operating over a range of pressures, as is typical in SFC. In the absence of actual phase equilibria data, simple mole fraction additivity methods used to obtain mixture critical parameters can result in considerable error and lead to inadvertent operation in the vapor-liquid region. More complex predictive methods utilizing equations of state[68,69] or surface fraction functions (Chueh and Prausnitz method)[70] generally provide more accurate estimates of the true critical parameters. These considerations are important when pressure programming methods are used, but are of lesser importance when relatively high isobaric pressures are used.

The chromatographic behavior observed for a fluid mixture can also be used to discern whether a single-phase or a two-phase system exists under a specified set of operating conditions. This is illustrated with the open tubular column SFC-MS chromatograms of a coal tar shown in Figure 1.7. These separations were obtained at three different temperatures, but otherwise under identical operating conditions, using a 10% (v/v) mixture of isopropanol in propane mobile phase. At 120°C (Figure 1.7A), the components were poorly resolved and eluted close together. When the temperature was raised to 130°C (Figure 1.7B), the components were much better resolved and eluted over a wider pressure range. At 150°C (Figure 1.7C), only slightly improved performance was obtained. This behavior can be explained by the existence of a subcritical liquid with strong solvating properties at the lower temperature that eluted the components close together. Under such conditions, pressure programming is of relatively little value because density does not change substantially. At the intermediate temperature of 130°C, the fluid mixture was probably in a single-phase supercritical condition, and the expected higher chromatographic efficiency and an increasing solvating power with increasing pressure were observed. The differences between the chromatograms obtained at 130°C and 150°C can be attributed to the different fluid properties (viscosity and density at a given pressure) and analyte volatilities at the two temperatures.

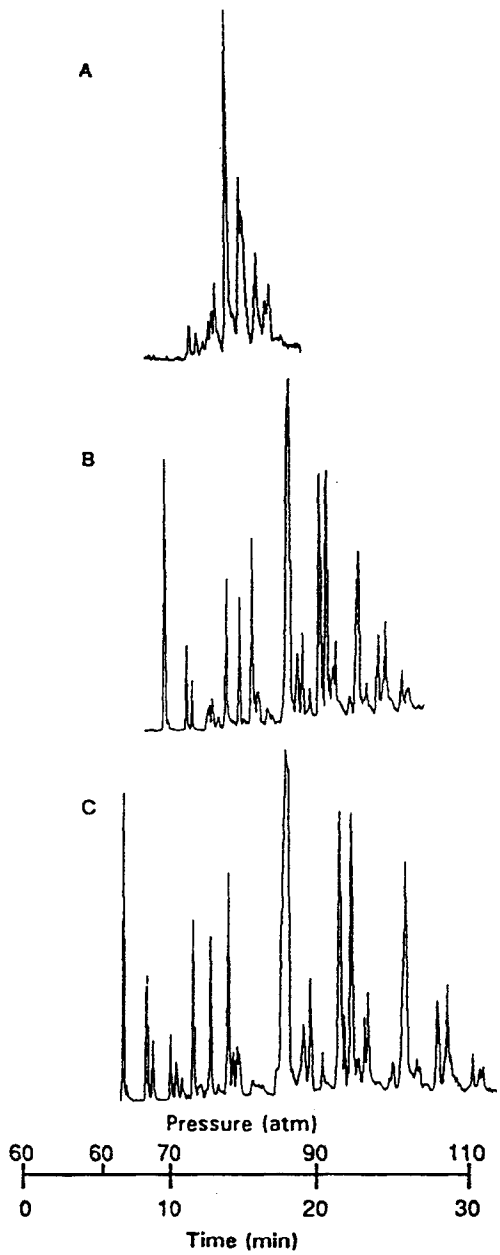

Figure 1.7. SFC/MS total-ion chromatograms of a coal tar extract obtained at (A) 120°C, (B) 130°C, and (C) 150°C. These chromatograms are aligned according to analysis time; apparent discrepancies are due to the delay in the start of data collection. Conditions: 10-m x 50-μm i.d. open tubular column, poly(phenyl)methylsiloxane stationary phase; 10% (v/v) isopropanol in propane; 60 atm for 5 min, 60 to 110 atm at 2 atm min^{-1} (reprinted with permission from Ref. 71).

The range of solvating power of practical supercritical fluids for SFC is of primary importance, and ultimately defines the limits of application. The solubility of analytes typically increases with density, and a maximum rate of increase in solubility with pressure is generally observed near the critical pressure, where the rate of increase of density with pressure is greatest.[72] There is often a linear relationship at constant temperature between log[solubility] and fluid density for dilute solutions of nonvolatile compounds (up to concentrations where solute-solute interactions become important). At constant pressure, when solute volatility is extremely low, and at densities less than or near the critical density, increasing temperature will typically decrease solubility.[73] However, solute entrainment in the fluid may increase at sufficiently high temperatures, where solute vapor pressure also becomes significant. Under conditions of constant density, solubility generally increases with temperature. Thus, while the highest supercritical fluid densities (at constant temperature) are obtained near the critical temperature, the greatest solubilities and lowest chromatographic retention will often be obtained at somewhat lower densities, but at higher temperatures.

As with liquids, polar solutes are most soluble in polar supercritical fluids, although nominally nonpolar fluids can be remarkably good solvents for many moderately polar compounds.[58] Carbon dioxide, for example, can exhibit solvating properties at higher pressures, intermediate between liquid *n*-pentane and dichloromethane. A comparison of the effective solvent polarity of seven fluids as a function of reduced density is shown in Figure 1.8.[74] Solvent polarity is defined in terms of solvent polarizability (π^*) which was developed by Kamlet *et al.*[75] to correlate different solvent-solute interactions based on the solvatochromic effect of the solvent on the π-π^* electronic transition of probe solutes. In this plot, π^* contains terms to account for solvent polarity (*i.e.*, dipolarity) and polarizability, but does not include effects from potential hydrogen bonding interactions.[76] At equal reduced densities, the various fluids have quite different π^* values, indicating that there are large differences in their effective polarities/polarizabilities. Ammonia has the largest π^* value, which supports the fact that it is the most polar solvent. The solvatochromic method also demonstrates the variable solvent properties of a supercritical fluid as a function of density.

Many polar solvents would offer highly specific solvating power but have excessively high critical temperatures, precluding practical operation with current stationary phases. The thermostability limits of the analytes themselves can also be exceeded. This has generated

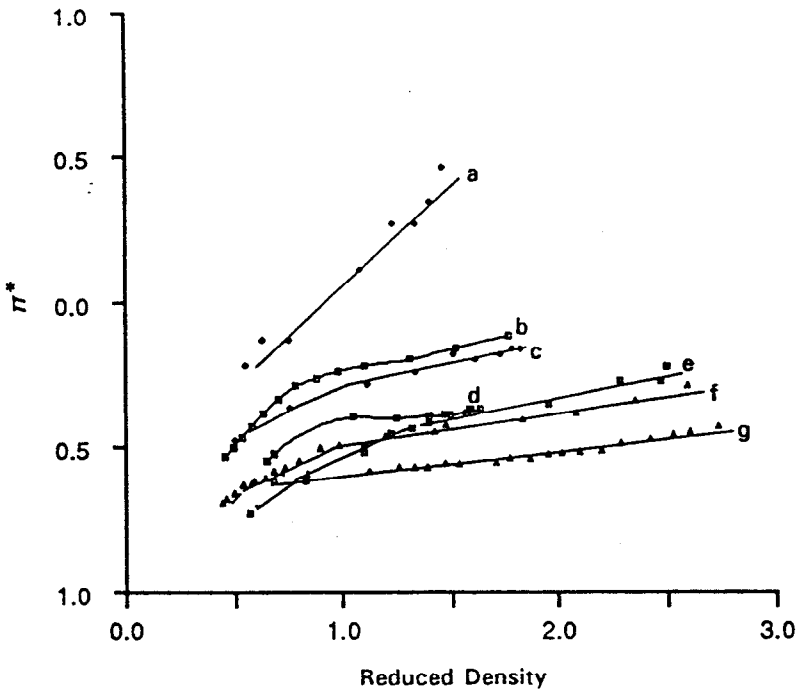

Figure 1.8. Solvent polarizability/polarity parameter (π^*) for various supercritical fluids as a function of reduced density at a reduced temperature of 1.03. Supercritical fluids: (a) NH_3, (b) CO_2, (c) N_2O, (d) Xe, (e) CCl_2F_2, (f) C_2H_6, (g) SF_6 (reprinted with permission from Ref. 74).

interest in mixed or binary fluid mobile phases that can have enhanced solvating power at lower critical temperatures. Solvatochromic studies suggest that such fluid mixtures have a net enrichment of polar modifiers in the cybotactic region (nearest neighbor solvation sphere) of the analyte.[77]

1.6 Relationship of SFC to GC and LC from Theoretical Considerations

Since supercritical fluids seem to combine many characteristics of liquids and gases, it is not surprising to find that SFC lies between GC and LC in many respects. Randall described SFC as ". . . a chromatographic technique that is a combination of and complementary to GC and HPLC."[78] Whether SFC redundantly combines characteristics of GC and LC, or fills a void between GC

and LC, is still a question for some (although opinions have been largely swayed toward the latter as more and more examples of problems uniquely solved by SFC have been published).

The link between SFC and GC is evident in the pioneering work of Sie, van Beersum, and Rijnders.[32] They proposed using a nonideal gas mobile phase, CO_2, at elevated pressures to increase interactions between the mobile phase and solutes. Their goal was to complement the solute volatility (and thus, the range of problems which could be addressed with GC) without raising the working temperature above the limits imposed by the stability of the solutes and stationary phase. Later, Sie and Rijnders measured volatility enhancement factors as high as 10^4 using supercritical *n*-pentane and isopropanol as mobile phase.[34] Also, Sie *et al.* noted that retention dropped rapidly as the mobile phase pressure was increased in the vicinity of the critical point, thus suggesting the possibility of eluting solutes too nonvolatile for ordinary GC.[32]

The bridge between SFC and LC was convincingly demonstrated by Lauer *et al.*[79] They showed that the logarithm of the capacity factor of solutes, measured at constant mobile phase density, changed linearly when plotted against reciprocal temperature, even as the critical isotherm is crossed (*i.e.*, in crossing from liquid to supercritical phase regions). Plots of the binary diffusion coefficients, measured at constant density, *vs.* reciprocal temperature, were also linear, even when crossing the isotherm.

The enhancement of solvation by the mobile phase can be quantitatively explained using an appropriate equation of state. The Peng-Robinson equation of state,[68] which works better than most for supercritical fluids, can be used to calculate the fugacity (activity) coefficient of the solute, which quantifies the solvating effect. The equation requires solute-mobile phase interaction parameters, which can be obtained from supercritical solubility measurements. Thus, for example, the maximum observed temperature dependence described earlier may be understood quantitatively using the Peng-Robinson equation.[80]

Martire and Boehm further linked GC, SFC, and LC with their "unified molecular theory of chromatography."[81] They developed a set of general equations applicable to GC, SFC, and LC which reduce to familiar retention expressions with the appropriate assumptions (see also Reference 82). Recently, it was demonstrated that an analysis using GC, SFC, and LC can be performed with each mode in series using a single column.[83] Ishii and Takeuchi have named this approach "unified fluid chromatography."[83] Both temperature and

pressure were adjusted so that elution could be programmed and modes could be switched.

There are no distinct boundaries between SFC and the other two techniques, even though the equipment necessary to perform each technique has its unique features. In the remainder of this section, the theory necessary to consider the benefits of choosing one mobile phase over another, and how the mobile phase choice affects the resulting chromatography, will be considered.

Basic premises. All forms of elution chromatography basically work in the same way regardless of the nature of the mobile phase. Solutes are partitioned between the mobile and stationary phases according to a unique partition coefficient (K_i) for each solute (i) in the sample. The value of K_i is given by

$$K_i = \frac{c_{i,s}}{c_{i,m}} \tag{1.1}$$

where $c_{i,s}$ and $c_{i,m}$ represent the concentrations (as mass per volume) of component i in the stationary and mobile phases, respectively. The distribution of each solute between the stationary and mobile phase is described by the capacity factor (k_i)

$$k_i = \frac{x_{i,s}}{x_{i,m}} = \left(\frac{c_{i,s}}{c_{i,m}}\right)\left(\frac{V_s}{V_m}\right) = \frac{K_i}{\beta} \tag{1.2}$$

where x represents the masses of components i in each phase, V_s and V_m are the volumes of the stationary and mobile phases, respectively, and β is the phase ratio of the column, that is, V_m/V_s.

When the column is not overloaded, the velocity (u_i) of each solute in the column will be

$$u_i = \frac{u}{(1 + k_i)} \tag{1.3}$$

where u is the velocity of the mobile phase. For solutes with similar capacity factors, the distance (or time) separating the peaks increases in direct proportion to the distance traveled along the column, while the peak widths increase in proportion to the square root of the distance traveled. These peaks are resolved from each other at the column exit if the column, as used, has enough efficiency and/or selectivity.

Peak broadening in open tubular columns. Ideally, peaks are narrow and near Gaussian shaped at the detector. The width of a Gaussian peak can be described by its standard deviation (σ). If the peak is broadened by several "independent" mechanisms, then its width is described by

$$\sigma = (\sigma_a^2 + \sigma_b^2 + \ldots)^{1/2} \tag{1.4}$$

where the subscripts (a, b, . . .) denote the individual broadening mechanisms.

The efficiency of a column is described by the number of theoretical plates (n) that it can provide under a particular set of nonprogrammed (*i.e.*, isothermal, isocratic, isobaric, etc.) conditions

$$n = \left(\frac{t_r}{\sigma}\right)^2 = 5.54\left(\frac{t_r}{w_{1/2}}\right)^2 \tag{1.5}$$

for Gaussian-shaped peaks where t_r is the time from the moment of injection required to elute the peak, and $w_{1/2}$ is the width of the peak measured at half its maximum height. (Note, σ and $w_{1/2}$ must be described in the same units as t_r.)

To consider peak broadening, we need the concept of the height equivalent of a theoretical plate (h). Empirically, h is determined by finding n from the peak width, knowing the length of the column (L) and using the relation

$$h = \frac{L}{n} \tag{1.6}$$

The value of h for an open tubular column can also be predicted using the Golay equation[84]

$$h = \frac{2\,D_m}{u} + \frac{d_c^2(1 + 6\,k + 11\,k^2)u}{96\,D_m(1 + k)^2} + \frac{2\,k\,d_f^2\,u}{3(1 + k)^2\,D_s} \tag{1.7}$$

which in its simplified form is given by

$$h = \frac{B}{u} + Cu = \frac{B}{u} + (C_m + C_s)u \tag{1.8}$$

The B term describes the fraction of h caused by longitudinal diffusion, and is equal to $2\,D_m$, where D_m is the diffusion coefficient of the solute in the mobile phase. The C terms describe the fraction

of h caused by resistance to mass transfer in the mobile and stationary phases (as denoted by the subscripts m and s). The C_m term results because of laminar flow through the open tube which gives rise to the characteristic parabolic velocity profile over the tube cross section. Solutes in the center of the flow move faster than solutes near the wall. Failure of the solute to rapidly diffuse in a radial direction (that is, resistance to mass transfer in the mobile phase) tends to keep the solute distributed on streamlines of differing velocity, thus broadening the peak. The C_s term describes the contribution to h caused by solute diffusion in the stationary phase. However, since this is usually negligible in open tubular columns (especially when thin coatings of stationary phase are used), and because it does not directly involve the effects of mobile phase choice, it can be ignored. Thus, C is essentially C_m. The value of C will be different for each solute, depending on the values of the individual capacity factors and diffusion coefficients (at least for those solutes with k values below about 10; the ratio of the two factors containing k approaches a constant value of 11 for increasing k, and can be considered practically constant for k values of 10 or greater). Thus, h and n for the column will be different for each solute. Factors which correct for mobile phase compressibility and peak broadening due to mobile phase decompression are unnecessary in this discussion. The inclusion of these factors would improve the accuracy of the equations, but would not change the final general conclusions.

By differentiation of Equation 1.8, it can be seen that the optimum mobile phase velocity (*i.e.*, the velocity that produces a minimum value for h and a maximum value for n) is

$$u_{opt} = \left(\frac{B}{C}\right)^{1/2} = \frac{4.2\ D_m}{d_c} \qquad (1.9)$$

for thin stationary phase films (where C_s is negligible) and values of k greater than 10 (indicating a strongly retained solute).

Substitution of u_{opt} into Equation 1.8 yields

$$h_{min} = 2(BC)^{1/2} = 0.9\ d_c \qquad (1.10)$$

under the same conditions.

Equations for packed columns. Similar equations can be derived for packed column SFC. The van Deemter equation for packed columns is given by

$$h = A + \frac{B}{u} + Cu \tag{1.11}$$

where the A term represents eddy diffusion or flow nonuniformity and is equal to $2\lambda d_p$, where λ is a structural factor describing the geometry of the packed bed, and d_p is the average particle diameter of the packing. For packed columns, the equation for h_{min} includes the A term.

$$h_{min} = A + 2(BC)^{1/2} \tag{1.12}$$

1.7 Relationship of SFC to GC and LC from Practical Considerations

The choice of mobile phase obviously will have a great effect on mass transport in a chromatographic process. This will have a direct bearing on analysis time and efficiency. The role of solute volatility or solubility in the mobile phase will certainly depend on the mobile phase. Selectivity and solute stability may also change with the choice of mobile phase. Finally, various system characteristics, such as column inertness and detector compatibility, are affected by the choice of mobile phase.

Efficiency, speed of analysis, and resolution. Equations 1.10 and 1.12 indicate that the only variables affecting the best value of h that can be expected from the column is the column diameter (open tubular) or the particle size (packed). This is possible for any mobile phase, as long as the optimum velocity for that mobile phase is used, according to Equation 1.9. Thus, the optimum efficiency is a column characteristic (although the efficiency realized can be spoiled by inappropriate parameter choices, bad connections, extracolumn volumes, poor injections, etc.). Therefore, the major effect of mobile phase choice is not reflected in optimum column efficiency, but in analysis time (the optimum velocity depends directly on the diffusion coefficient of the solute in the mobile phase chosen).

In practice, different column types and dimensions are preferred for use in GC, SFC, and LC. Open tubular columns are generally preferred for GC and SFC, although packed columns are also popular for SFC. In LC, only packed columns are commonly used. Open tubular SFC columns usually have inside diameters between 25 and 100 μm, while typical GC columns range from 100 to 530 μm (although GC is occasionally performed using columns as small as 50-μm i.d.). The maximum efficiency of a 50-μm i.d. column in SFC is usually not realized in practice because this would require longer analysis times than are normally acceptable. Therefore, it is a common practice to operate SFC columns well above the optimum velocity to exchange efficiency for shorter analysis times.

Since GC is more rapid and more efficient than SFC and LC, it should be the first choice from among the three separation techniques whenever it is applicable to the problem at hand. From a speed and efficiency standpoint, SFC should be the second choice. Finally, when neither of these techniques are applicable, LC should be selected.

For open tubular column LC, column diameters would have to be between 5 and 10 μm in order to provide practical analysis times, and extracolumn effects would be even more difficult to overcome than in SFC. These difficulties make open tubular column LC an unpopular choice, despite the high efficiencies possible with such narrow-bore columns.

The primary factors affecting h in packed columns are the mean particle diameter (d_p) and the velocity. LC works well with small-diameter packings because of the low compressibility of the liquid mobile phase. High head pressures and large pressure drops over the column are of no great consequence. Thus, modern microparticle LC columns (packed with 3- to 10-μm diameter particles) can deliver reasonable efficiencies and reasonable analysis times. Pressure drops on these columns are much smaller with supercritical fluid mobile phases because of their low viscosities compared to liquids. Therefore, even though supercritical fluids are compressible, the relatively low pressure drops required to achieve optimum velocity make packed-column SFC with microparticle packings successful.

GC is not generally practical with microparticle columns, although the pressure drop would be near that of the SFC case at the same velocity (since viscosities are similar); this velocity is far too slow for a gaseous mobile phase. If operated at optimum velocity, the pressure drop would be very large. It is much more practical in packed column GC to use larger particles to reduce the pressure drop,

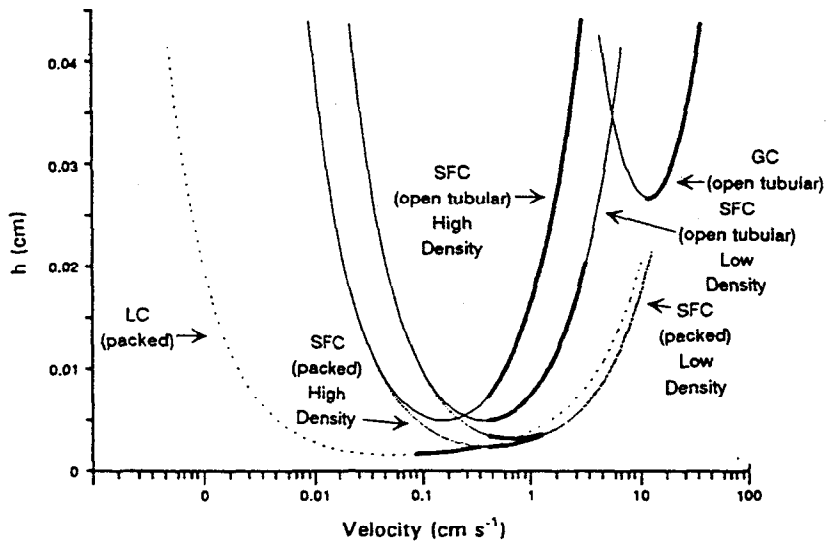

Figure 1.9. Efficiency as a function of mobile phase velocity. Commonly used efficiency-velocity ranges are highlighted. Packed column conditions: 5-μm particles, values calculated from the Knox equation with A = 1, B = 2, C = 0.05 (for LC), and C = 0.5 (for SFC). Open tubular column conditions: 50-μm i.d. (SFC), 300-μm i.d. (GC), values calculated from the Golay equation with k = 5. Values for D_m were 10^{-5} cm^2 s^{-1} for liquid, 2 x 10^{-4} cm^2 s^{-1} for high density supercritical fluid (100°C), 5 x 10^{-4} cm^2 s^{-1} for low density supercritical fluid (100°C), and 10^{-1} cm^2 s^{-1} for gas.

and then increase the column length to achieve the needed efficiency (or, better yet, just use an open tubular column).

Considering all pertinent dimensions and typical values of diffusion coefficients for the various mobile phases, van Deemter-type efficiency curves for the various combinations of parameters are plotted in Figure 1.9. The Golay equation was used for the open tubular columns, and the Knox equation[85] was used for the packed columns. The commonly used regions of these curves are highlighted.

The validity of the Knox equation under SFC conditions has not been experimentally well established, especially at high velocities. Hence, the packed column curves in Figure 1.9 were not extended much past the normal velocity ranges. The earlier approach of ignoring resistance to mass transfer in the stationary phase (*e.g.*, as in Reference 78) is also probably incorrect for microparticle packings

with high surface areas and appreciable solute retention *via* adsorption. This was a more correct assumption in early SFC work in which GC packings were used. The importance of considering resistance to mass transfer in the stationary phase when using LC-type packings for SFC can be summarized as follows: if resistance to mass transfer in the stationary phase is important for a particular packing used under LC conditions, then its relative contribution to peak broadening will be even higher with a supercritical fluid mobile phase. If stationary phase mass transfer determines h at high velocities in both LC and SFC operation with a particular packing, then the efficiency curves for the LC and SFC modes will tend to merge at high velocities. This indicates that to achieve large benefits in analysis time with packed column SFC, the packings must operate as much as possible with a (fast) partition mechanism and that the (slower) adsorption/desorption retention mechanism must be minimized.

Figure 1.9 is potentially deceptive in terms of assigning merit to the different techniques. The value of h and its corresponding velocity are of fundamental importance, but are meaningless in a

Table 1.3. Efficiency and Analysis Time Ranges for Various Chromatographic Techniques

Technique[a]	Velocity range (cm s^{-1})	Efficiency range (n)	Efficiency/-time range (n s^{-1})	Elution time range[b] (min)	Practical analysis time range[c] (min)
LC (packed)	0.1 - 0.4	5,300 - 8,500	14 - 35	2.5 - 10	0.5 - 60
SFC (packed)					
Low density	0.5 - 1.5	3,300 - 3,700	31 - 79	0.7 - 2	0.3 - 30
High density	0.5 - 1.5	3,500 - 5,100	42 - 83	0.7 - 2	0.3 - 30
SFC (open tubular)					
Low density	0.5 - 4	50,000 - 221,000	18 - 33	25 - 200	5 - 90
High density	0.5 - 4	20,000 - 137,000	11 - 13	25 - 200	5 - 90
GC (open tubular)	15 - 50	64,000 - 112,000	93 - 180	6 - 20	1.5 - 60

[a]LC (packed): 10-cm column length with 5-μm packing.
SFC (packed): 10-cm column length with 5-μm packing.
SFC (open tubular): 10-m column length with 50-μm i.d.
GC (open tubular): 30-m column length with 300-μm i.d.
All except the last column are calculated for nonprogrammed elution with k = 5.
[b]Nonprogrammed conditions, for a solute with k = 5.
[c]Typical programmed conditions.

practical sense without consideration of the column length. The efficiency of the entire column is a more practical consideration. Table 1.3 lists the efficiency ranges of typical columns in each technique by combining the results of the calculations leading to Figure 1.9 with typical values of column lengths and the normal velocity range. These numbers still do not tell the whole story, because analysis time has not been considered. Using k = 5 and considering the same velocities and column lengths, the analysis time range for each technique was calculated and is also listed. Finally, the techniques can be compared on the basis of efficiency per unit time. This type of information is often used inappropriately to justify the selection of a particular technique, and must be interpreted with caution. Efficiency per unit time gives the power of a technique to generate theoretical plates, but says little about the ability of a column to separate peaks.

Resolution between two near equally retained peaks is simply the difference in elution times divided by the average peak widths measured at the baseline. In terms of chromatographic parameters, the resolution (R_s) is

$$R_s = \frac{n^{1/2}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{k_2 + 1}\right) \qquad (1.13)$$

where α is the column selectivity factor for the two peaks ($\alpha = k_2/k_1$ where the peak numbers are assigned such that $k_2 > k_1$). From this equation, it is clear that resolution depends on column efficiency, selectivity, and solute retention. If n or k approach zero, or if α approaches 1, the resolution will be lost, regardless of what the values of the other parameters might be.

The description so far has dealt only with nonprogrammed elution. Programming further complicates the situation. If the last peak of interest is eluted during a post-program hold period, then the analysis time with any chromatographic technique will be roughly the time of the program up to the final hold, plus $(1 + k_2)L/u$. Equation 1.13 still applies under programmed conditions, except that the constant k values must be replaced with dynamic values which reflect the effect of the program. The analysis time can be shortened and the peaks narrowed in any programmed chromatographic technique by using a faster program rate. However, this is analogous to eluting peaks with a lower average value of k, which, if reduced sufficiently, will lower the resolution of the technique and merge the peaks even though they are narrow.

Volatility or solubility. Among the partition chromatography methods to choose from in practical laboratory situations, GC is most rapid, delivers the highest efficiency, and has the greatest efficiency per unit time. However, GC is limited to thermally stable solutes with vapor pressures of a few torr, and to thermally stable stationary phases.

The partitioning of solutes into a supercritical fluid at a particular temperature is usually enhanced over what would be observed due to "ordinary" vapor pressure in GC. This enhancement is attributed to the solvating properties of supercritical fluids. A rigorous treatment of this phenomenon is beyond the scope of this book, but several excellent summaries are available (for example, References 74 and 86). For purposes of this discussion, it can be stated that the partition process in GC follows the van't Hoff equation

$$\ln K = \frac{\Delta G^{\theta}}{RT} \tag{1.14}$$

or, upon application of Equation 1.2,

$$\ln k = -\frac{\Delta G^{\theta}}{RT} - \ln \beta \tag{1.15}$$

where ΔG^{θ} is the free energy change associated with the partitioning of a solute between the stationary phase and an inert mobile phase, R is the gas constant, and T is the absolute temperature. In GC, the free energy change is essentially the free energy of solution of solute in the stationary phase. There is no significant interaction between the solute and the mobile phase. However, whenever the solute can be at least partially solvated by the mobile phase, the overall effect is a reduction in magnitude of the net free energy change of the partition process and a reduction in the values of K and k. The mobile phase now competes with the stationary phase for the solute, which lowers the retention. Thus, the first benefit of using a solubilizing supercritical fluid in place of an inert gas mobile phase is a reduction in solute retention at a given temperature. This, in turn, means that the molecular mass range can be substantially increased, or the temperature required for elution can be reduced. Thus, thermally labile materials may be successfully analyzed with SFC (if the fluid chosen has a low critical temperature) when GC would fail. These benefits could be combined by using a temperature intermediate between that required by GC and the critical temperature of the supercritical fluid.

The use of a liquid mobile phase can reduce retention even further by virtue of even stronger solvation. Of the three types of mobile phases considered, polar liquids are the only mobile phases suitable for dissolving common salts. Very high molecular mass solutes, such as proteins and large polymers, usually require liquid solvents and, thus, can only be analyzed by LC techniques. Most SFC work has been done, so far, for low-to-medium molecular mass and relatively nonpolar solutes. Polystyrenes with molecular masses up to 600,000 daltons have been successfully eluted from an SFC column.[87] However, the limits of SFC in terms of the permissible range of solute polarity and molecular mass are not clear.

In some circumstances,[88] there is a quantitative inverse relationship between the capacity factor and the solubility (s) of the solute in the mobile phase

$$s = \frac{C}{k} \tag{1.16}$$

where C is a constant for the solute, column, and temperature; it is independent of the mobile phase; and it has a temperature dependence of

$$C = A\, e^{B/T} \tag{1.17}$$

where A and B are constants. This relationship provides a route for the measurement of both liquid and supercritical fluid solubilities.

Selectivity. Selectivity in GC is determined exclusively by the stationary phase, since the mobile phase is only a carrier and has no interaction with the solutes. Since the selectivity factor is merely the ratio of the k values for two peaks, it follows from Equation 1.15 that the selectivity in GC is determined simply by the differences in heats of solution of the two solutes in the selected stationary phase (assuming the entropy change for partition of the two solutes is similar).

In LC, large adjustments in selectivity are possible by making compositional changes in the mobile phase, as well as by changing the stationary phase. Selectivity predictions are more empirical than in GC, because both phases interact with the solutes, and the retention process often has adsorption contributions.

In SFC, both the stationary and mobile phases can be varied as in LC. However, there are constraints on the mobile phase composition since the critical parameters are a function of the composition. The selectivities in SFC sometimes resemble GC and

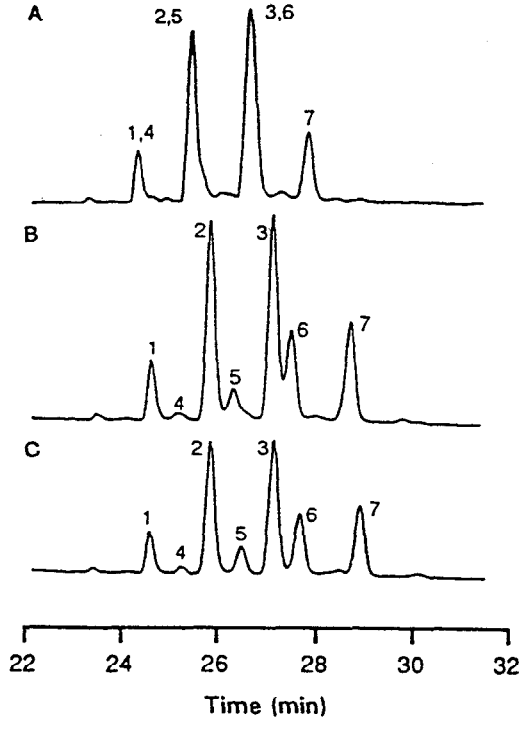

Figure 1.10. Selected segments of SFC chromatograms from a complicated sample, showing the effect of temperature on selectivity at (A) 140°C, (B) 160°C, and (C) 170°C. Two families of unknown peaks (1-3 and 4-7) overlap with some coelution at 140°C, but are resolved at 170°C. Conditions: 10-m x 50-μm i.d. open tubular column, poly(30% biphenyl)methylsiloxane stationary phase; CO_2; 5 atm min^{-1}, FID.

other times resemble LC. This is dependent on the chromatographic conditions, the mobile phase, and the solutes.

There is one noteworthy difference between selectivity adjustment in SFC and in GC and LC. Since mobile phase strength in SFC is a function of both temperature and density (for a fixed mobile phase composition), there are various combinations of temperature and density capable of eluting a particular solute with a desired value of k. However, if we manipulate temperature and density while keeping the k value of one solute fixed, there is no guarantee that the k values of other solutes will also stay fixed. In fact, shifts in relative retention are common, especially if the solutes contain different functional groups.[89] Thus, the selectivity can be changed to some extent by adjustment of the separation temperature.

This effect results from the unequal changes in solubilities of different solutes in the mobile phase as the temperature is varied. It is often possible to reverse the elution order of two close peaks or to split a merged pair. An example of how a relatively small temperature change can make a dramatic improvement in selectivity is shown in Figure 1.10.[90] This should usually be done for fine-tuning after selection of the best column/mobile phase combination for a particular separation.

Solute stability. SFC (with CO_2 mobile phase) can be performed at temperatures as low as about 35°C. Obviously, thermally labile solutes which cannot survive a GC separation at least have a chance of remaining intact after an SFC or LC separation, which may be at significantly lower temperatures. Chemical derivatives of solutes do not require the degree of thermal stability for SFC or LC analysis that is necessary for GC. Even compounds that may appear to be thermally stable (for example, when analyzed by thermogravimetric analysis) may still cause problems on certain columns. Reactions of solutes with active sites, or with impurities adsorbed on the column, are usually reduced at lower temperatures.

Column inertness. The silica surface in an open tubular column has no function other than to support the stationary phase. Thus, it is desirable to make the surface as inert as possible to minimize or eliminate undesirable interactions with the solutes. This is accomplished by extensive deactivation, as described in Section 2.2. Furthermore, the total surface area is rather small because the inside walls are smooth.

In contrast, many silica-based LC packings make use of interactions with the silica support in combination with the bonded stationary phase to achieve a particular degree of selectivity. Some adsorption/desorption equilibria occur with most bonded-phase, porous packings in addition to the solute interactions with the bonded phase. While this may be of benefit in LC, the slow adsorption/desorption kinetics cause problems in SFC because of the higher mobile phase velocities. The use of wide-pore silicas (with lower surface areas), polymer-coated silica packings, and polymer-based packings are all better alternatives for SFC.

Detection. From the detection viewpoint, SFC has an advantage over the other column chromatographic techniques. It can be interfaced to most GC and LC detectors, with only a few fairly

obvious limitations. For example, doping of inorganic mobile phases with organic solvents cannot be done when it is intended to detect organic solutes with a flame ionization detector. An electron capture detector cannot be used with electron-capturing mobile phases (*e.g.*, fluorinated hydrocarbons) or when electron-capturing impurities are present in the mobile phase. Also, strongly absorbing mobile phases cannot be used with spectroscopic detectors. The commonly used supercritical fluid mobile phases, particularly CO_2, have a tremendous range of detector compatibility. Specific information on the use of various detectors is given in Chapter 4.

1.8 General Utility of SFE Off-Line and On-Line with Chromatography

Extractions using supercritical fluids, whether off-line or on-line, are attractive for a number of reasons when compared to conventional liquid extractions. Their lower viscosities and higher solute diffusivities improve mass transfer from solid or liquid matrices and, thus, decrease the overall time needed for extraction. By increasing the density, the solvent strength of the supercritical fluid usually increases. Therefore, conditions can be optimized for the extraction of a specific solute or class of solutes from a complex matrix by changing the extraction pressure or temperature. Temperature or pressure changes, when near the critical point of the supercritical fluid, can change solute solubilities by as much as a factor of 100, or even 1,000. By using different supercritical fluids, selective extractions can be achieved for different solutes. Moreover, the use of fluids that have low critical temperatures (*i.e.*, CO_2, N_2O, and SF_6) allow extractions under thermally mild conditions, protecting thermally labile components.

Using certain supercritical fluids that are gases at room temperature, off-line component collection or concentration is greatly simplified. Because these supercritical fluids undergo expansive (*i.e.*, Joule-Thompson) cooling during decompression, even volatile components can be quantitatively and efficiently collected off-line in solvents. It is also possible to directly interface SFE with analytical chromatography. Recent reports have demonstrated the potential of using SFE as an alternative to time consuming, less efficient, and less quantitative liquid solvent extraction techniques. Specific solutes, ranging from environmental priority pollutants to spices and

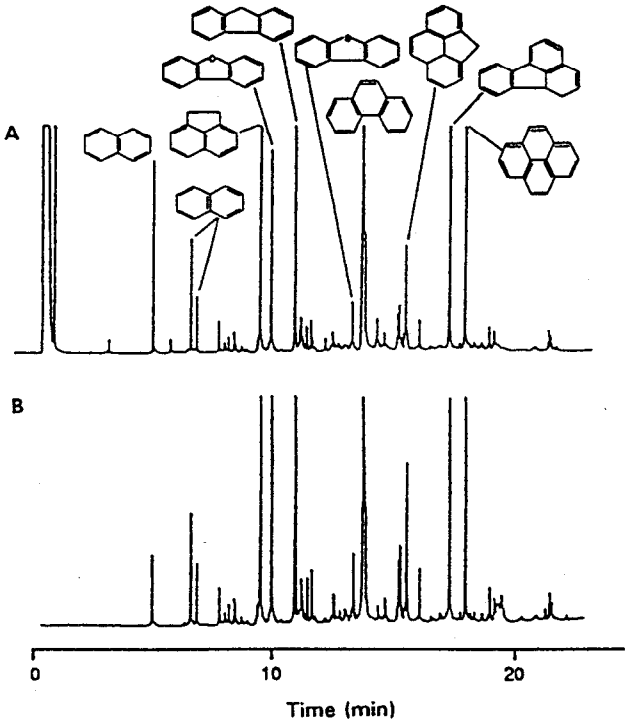

Figure 1.11. GC chromatograms of spiked river sediment extracts analyzed by (A) on-column GC injection and (B) on-line SFE/GC analysis. SFE conditions: 10-mg sample; N_2O; 300 atm; 45°C; 10 min. GC conditions: 30-m x 0.32-mm i.d., poly(5% phenyl)methylsiloxane stationary phase (d_f = 1 μm); 100°C to 320°C at 8°C min^{-1}; FID (reprinted with permission from Ref. 91).

fragrance components, have been qualitatively and quantitatively extracted.

The advantage of off-line SFE is that extraction effluents can be transferred to appropriate collection solvents and analyzed using a variety of analytical techniques (*e.g.*, GC, SFC, LC, MS, NMR, and FTIR). For many samples, collection solvents are not necessary and, as the supercritical fluid effluent vaporizes, the extracted solutes can be quantitatively deposited onto a variety of surfaces (*e.g.*, KBr disc, MS direct probe, NMR tube, or empty autosampler vial). Because of the ability of SFE to rapidly and efficiently remove target solutes from a variety of matrices, off-line SFE can be very effective as a sample cleanup and concentration tool. By the addition of appropriate standards to the sample matrix or to the collecting solvent, quantitative results of recoveries and concentrations can be obtained.

The features of directly coupled SFE to GC or SFC are that no sample handling is required between the extraction and the GC or SFC separation stages, and that extraction effluents can be quantitatively and reproducibly transferred for on-the-fly analyses. When employing an FID, there is no detector response when using extremely pure inorganic supercritical fluids for extractions. This permits the determination of solutes of interest which are often masked by liquid solvents when using conventional extraction techniques. Moreover, when modifiers are used to augment the solubilizing power of primary supercritical fluids, additional components can be analyzed. While SFE/GC is limited to volatile organic compounds, SFE/SFC can extend the analysis to higher molecular mass materials.

The ability of directly coupled SFE/GC to yield good quality chromatograms with minimum extraction and analysis times is demonstrated in Figure 1.11. As can be seen, comparable chromatographic efficiency is obtained with SFE sample introduction as with conventional syringe injection. Since the entire sample can be transferred from the extraction vessel to the GC column, only very small samples are needed to achieve low detection levels.

The use of SFE for the selective extraction of specific solutes or classes of solutes from complex matrices can be seen in Figure 1.12 by the SFE/SFC analysis of coal tar pitch with supercritical CO_2 at various pressures.[92] As the pressure was increased, higher molecular mass components were extracted. Temperature can also be used to alter extraction selectivities.

Figure 1.13 demonstrates the selectivities obtained when using two different fluids, SF_6 and CO_2, for the SFE/GC analysis (actually the conditions were subcritical) of an aromatic and alkane test mixture. With SF_6, only alkanes (*i.e.*, decane, dodecane, and pentadecane) were selectively extracted, regardless of pressures, temperatures, or extraction durations. With CO_2, under identical extraction conditions, both alkanes and aromatics through chrysene were extracted.

Using modifiers, the solvent strengths of supercritical fluids can also be greatly enhanced for the extraction and chromatography of polar or high molecular mass solutes. Most modifiers give responses in the FID, requiring the use of other detectors. Using a UV detector, 1 ppm of quinclorac (3,7-dichloro-8-quinoline carboxylic acid) was selectively determined in soil with a total sample preparation and analysis time of 30 min.[94] The conventional sample preparation method involved a time-consuming reflux with aqueous base, solvent extraction, and methylation before GC/MS or GC/ECD analysis.

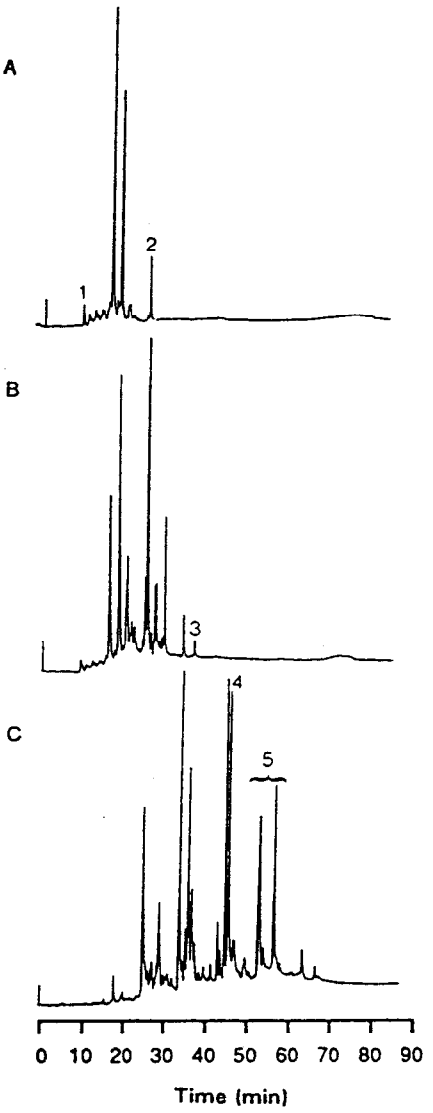

Figure 1.12. SFE/SFC chromatograms of a coal tar pitch extracted at (A) 70 atm, (B) 100 atm, and (C) 200 atm. SFE conditions: CO_2; 43°C; 1 h. SFC conditions: 10-m x 50-μm i.d. open tubular column, poly(30% biphenyl)-methylsiloxane stationary phase (d_f = 0.25 μm); CO_2; 110°C; density program from 0.25 g mL^{-1} to 0.74 g mL^{-1} at 0.006 g mL^{-1} min^{-1} after an initial 20-min isopycnic period; FID. Peak identifications: (1) naphthalene, (2) phenanthrene, (3) pyrene, (4) chrysene, (5) benzopyrenes and benzofluoranthenes (reprinted with permission from Ref. 92).

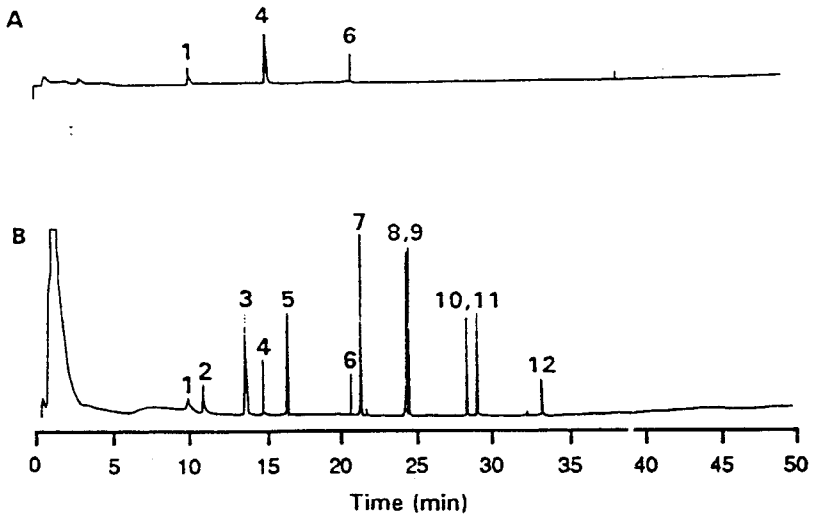

Figure 1.13. SFE/GC chromatograms of aliphatic and aromatic hydrocarbon test mixture obtained by (A) SF_6 extraction and (B) CO_2 extraction. SFE conditions: sample adsorbed on alumina; 375 atm; 25°C; 30 min. GC conditions: 50-m x 200-μm i.d. open tubular column, polymethylsiloxane stationary phase (d_f = 0.5-μm); 30°C for 8 min, then to 310°C at 7°C min^{-1}; FID. Peak identifications: (1) *n*-decane, (2) butylbenzene, (3) naphthalene, (4) *n*-dodecane, (5) 1-methyl naphthalene, (6) *n*-pentadecane, (7) fluorene, (8) phenanthrene, (9) anthracene, (10) fluoranthene, (11) pyrene, (12) chrysene (reprinted with permission from Ref. 93).

The current major limitation to on-line SFE/chromatography is the unavailability of autosampling devices. At present, samples must be analyzed individually, requiring the constant attention of the analyst. The tremendous potential of this technique will only be realized when reliable autosamplers become available.

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